90-Day Mouse Study To Evaluate Key Events In 1,4-Dioxane-Induced Liver Tumors

Sponsored by The American Chemistry Council's 1,4-Dioxane Panel
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Background

Ex. 5 - Deliberative Process

EPA's 2013 IRIS assessment established an oral cancer slope factor for 1,4-dioxane based on evidence for liver tumors in male and female mice and rats in studies conducted by the National Cancer Institute (1987) and the Japan Bioassay Research Center (JBRC) in 1990.[1] Although Health Canada, the Netherlands RIVM, and Australian NICNAS have concluded that the animal tumors are the result of a cytotoxic (threshold) response, the IRIS assessment concluded that the evidence for a cytotoxic mechanism of action (MOA) was not conclusive. The IRIS assessment defaulted to a linear, multistage approach to calculating the cancer risk that assumes no threshold.

Based on its analysis, EPA estimates that an acceptable level of 1,4-dioxane exposure is between 0.35 and 35 micrograms per liter (µg/L) in water – depending on the risk threshold used. This estimate compares to a value of 350 µg/L based on a cytotoxic MOA.

The IRIS assessment agreed that an MOA hypothesis involving cytotoxicity and subsequent regenerative liver cell proliferation has "some" support from data indicating that 1,4-dioxane is only weakly mutagenic and acts as a tumor promoter in rat livers. EPA further acknowledged that the dose-response and temporal data support the occurrence of cell proliferation prior to the development of liver tumors in the rats. The IRIS assessment rejected the cytotoxic MOA, however, noting the following concerns –

- Data regarding a plausible dose response and temporal progression from cytotoxicity to cell proliferation to eventual liver tumor formation are not available; and
- Conflicting data from mouse bioassays suggest the formation of tumors in the absence of cytotoxicity.

In late 2016, 1,4-dioxane was identified as a priority chemical under the amendments to the Toxic Substances Control Act (TSCA) passed that summer. The TSCA amendments require EPA to complete a risk evaluation by the end of 2019 for those conditions of use of 1,4-dioxane that the Agency deems appropriate. As part of this evaluation, EPA is required to use the best available science to conduct a weight of evidence review of the health effects of the chemical. The health evaluation under TSCA is likely to replace the 2013 IRIS assessment, in view of the

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[1] EPA derived the cancer slope factor from the JBRC data for liver tumors in female mice because they were the most sensitive species and sex for the endpoint. Questions have been raised as to whether mice are the appropriate model based on their inherent sensitivity to developing liver tumors. Concern also exists about the overall health of the mice used in the JBRC study given the low survival rate in the control group (58%).

new requirements for systematic review and evidence integration – something not previously conducted by IRIS for 1,4-dioxane, but now required by current IRIS weight-of-evidence approaches.

Review of NCI and JBRC Bioassays

During the peer review of the IRIS assessment, it was suggested that other histological findings may not have been recorded in the presence of liver tumors in the NCI and JBRC studies which may have led to underreporting of evidence of cytotoxicity. It was thought that a reread of slides from the NCI and JBRC bioassays could help explain the inconsistency in the results between the rat and mouse data. Dourson *et al.* (2014) subsequently reevaluated the mouse liver slides from the 1978 NCI bioassay and reported evidence of dose-related, non-cancer changes in the mouse livers, but noted that the changes were not as apparent in female mice. They identified specific key events in a cytotoxic MOA for liver tumors –

1	2	3	4	5
Accumulation of parent compound	Liver cell changes & death	DNA synthesis	Regenerative cell proliferation	Promotion of liver tumors

Dourson *et al.* (2017) subsequently reviewed translated reports from the JBRC studies in hopes of resolving the inconsistencies in the mouse data between the Japanese bioassay, the NCI slide review, and a 13-week study also conducted by JBRC. They were not able to resolve the inconsistencies, however, in the absence of a sufficient number of histopathology slides from the JBRC studies.

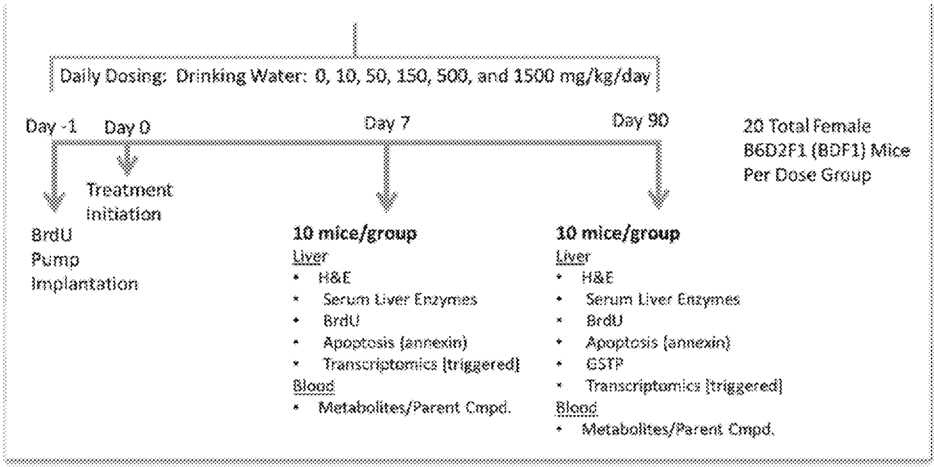
MOA Confidence Analysis

Using the information developed by Dourson *et al.*, ACC compared the weight of evidence for a cytotoxic MOA to the default mutagenic MOA using a confidence scoring approach proposed by Becker e*t al.* (2017). This comparison indicates that the overall pattern of observations is far more consistent with a cytotoxicity response than with a mutagenic one and that the likely operative MOA for rodent liver tumors is cytotoxicity.

Questions remain, however, about the MOA, the progression from non-cancer liver effects to tumors, and the inconsistency in the mice data. In the absence of additional data to address such questions, and in light of the significant time constraints on EPA's evaluation, the upcoming TSCA review would benefit from additional Key Event information to support a tumor promotion MOA with dosimetry and pharmacokinetics allowing improved estimates of points of departure and toxicity values.

Study Design

The following figure lays out the study design for the conduct of the 90-day study.



This liver MoA study represents a comprehensive approach, based on the current limited key event and mode-of-action data, to investigate the treatment-related liver effects known to result from 1,4-dioxane administration.

The proposed study design maximizes the interpretation of previous knowledge of the known effects of the test material in an effort to elucidate plausible MoA and key events for mouse liver tumors. Importantly, this experimental approach is meant to rule-out as well as rule-in many of the known potential MoA for the generic induction of mouse liver tumors; however, the experimental design is tailored to the specific, known toxicology of 1,4-dioxane, e.g, the metabolic saturation kinetics whereby the parent compound drives the liver tumor promotion response.

The in-life portion of the study is proposed to be a 90-day exposure period, with a separate satellite group of animals sacrificed after 7 days of treatment. Any samples not analyzed as part of the original study protocol for interim collection times will be stored for potential future investigation in a manner conducive to subsequent triggered analysis.

The study will use B6D2F1 (BDF1)/Crl female mice (n=10/dose/group) to closely model the carcinogenicity study (which was performed in Crj:BDF1), and 1,4-dioxane will be administered in the drinking water. The 1,4-dioxane doses were selected to bracket the biological effect (tumors) as well as the presumptive toxicokinetic transition points (0, 10, 50, 150, 500, and 1500 mg/kg/day). Female mice were selected as they were more sensitive to the induction of hepatic tumors; however, estrous cycling is known to affect hepatic proliferation, therefore efforts will be taken to minimize and characterize that potential confounding factor on data interpretation.

The data from each of the end points (detailed below) will be used in a key event and dose-response assessment to further characterize the operant MoA and, ultimately, identify a point-of-departure for the molecular and tumorigenic response. The identified end points to be collected are meant to characterize the liver status at the cell, tissue, and organ level. Importantly, this work will also evaluate the dosimetry and toxicokinetics of the administered 1,4-dioxane. Individual cell apoptosis status can be measured by annexin V and/or caspase 3/7 activation. Hepatic parenchymal tissue/organ status (damage) can be measured by routine serum clinical chemistry parameters (e.g., ALT, AST, and GGT) and correlated with histopathological evaluation (H&E). Characterization of preneoplastic hepatic foci can be performed by immunohistochemical staining and analysis for glutathione S-transferase, placental isoform (GST-P). In addition, by using an osmotic pump to administer BrdU for a set duration (typically ~7 days) prior to tissue collection, a robust assessment of hepatic proliferation (i.e., DNA synthesis) can be undertaken. Because the proliferative index of hepatocytes is relatively low, use of a non-accumulating marker of proliferation such as Ki-67 results in only a limited window of analysis. Further, a pulse-type experiment with BrdU ip injection may miss a treatment-related induction of proliferation. As previously mentioned, it is well-known that estrous cycle in female mice alters the hepatocyte proliferative status, therefore particular attention will be paid to the specifics on pump implantation and necropsy.

RNA Sequencing

Lastly, the use of RNA gene expression analysis allows the identification (or exclusion) of specific, known MoA that may be relevant in, for example, nuclear receptor-mediated hepatocarcinogenesis. Furthermore, transcriptomic analysis by a whole-genome approach allows the identification of relative biological activity (irrespective of a specific, known MoA) to determine clear points-of-departure. These data can then be correlated to the other cell, tissue, organ, and animal-specific data such as toxicokinetic parameters to further characterize the biological response.

In conclusion, the proposed study has been designed to elucidate the MoA for murine hepatic tumors. Characterization of the MoA along with mechanistic determination of points-of-departure for critical key events (with respect to apical tumor response) will further support the risk assessment and management of 1,4-dioxane.